JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1981, p. 279-282
0095-1137/81/020279-04$02.00/0

Vol. 13, No. 2

Rapid Method for Identification of Group B Streptococci in Neonatal Blood Cultures

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A rapid technique used for the identification of *Streptococcus agalactiae*, Lancefield group B, from the blood cultures of two neonatal infants is reported. The method utilized the Phadebact Streptococcus Test System (Pharmacia Diagnostics, Piscataway, N.J.) and the supernatant from 13- and 14-h blood cultures. Additional studies with simulated neonatal blood cultures revealed that this method was reproducible. Additional studies also revealed that some nonspecific agglutination did occur, which could not be eliminated with dithiothreitol, but was visibly reduced by treatment with soluble staphylococcal protein A.

The importance of *Streptococcus agalactiae*, Lancefield group B, in association with human disease is well documented (3, 9). The group B streptococci are considered to be one of the most common causes of sepsis and meningitis among neonates (4, 7), and roughly one-third of neonate meningitis is caused by group B streptococci (10). It has been estimated that 12,000 to 15,000 infants annually in the United States will develop group B streptococcal infections of a serious nature with a mortality rate of 30 to 50% (2, 3). The acute and often unexpected nature of this infection accompanied with the expected mortality rate emphasizes the need for a rapid technique to identify the group B streptococci when suspected in neonatal blood and spinal fluid specimens. A technique is described here which allowed the identification of group B streptococci in the blood cultures of 2- and 3-week-old infants within 13 and 14 h, respectively, after specimen collection.

MATERIALS AND METHODS

Patient 1. A 3-week-old female infant was admitted to the emergency ward with history of fever, strange cry, twitching movements of the right arm, and general tremors of the body 1 day before admission. On the day before admission, the infant had one episode of vomiting. Birth weight was 3260.25 g (7 lbs. 3 oz.) with a normal, uncomplicated delivery and normal neonatal course.

Physical examination showed a 4195.8 g (9 lbs. 4 oz.) infant who was irritable with a high-pitched cry and light skin mottling. Rectal temperature was 38.9°C (102°F). The anterior fontanelle was full but not under pressure. Nuchal rigidity was not present. Blinking movement of the right eye was noted.

Cerebral spinal fluid examination showed 3,475 cells per mm^3, 100% neutrophiles, 187 mg of protein per dl, and 6 mg of glucose per dl. Gram staining of a centrifuged specimen revealed gram-positive cocci in pairs and short chains. The leukocyte count at admission was 3.5×10^3 cells per mm^3, hemoglobin was 13.1 g/dl, and the platelet count was 306×10^3 cells per mm^3, with 18% segmented neutrophiles, 38% bands, 25% lymphocytes, 2% monocytes, 1% eosinophiles, 13% metamyelocytes, and 3% myelocytes. The leukocyte count on the following day rose to 18.7×10^3 cells per mm^3, with a differential count of 11% segmented neutrophiles, 51% bands, 32% lymphocytes, 5% monocytes, 1% atypical lymphocytes, and 1 nucleated erythrocyte.

The patient was initially treated with intravenous ampicillin (250 mg) every 6 h and intramuscular gentamicin (10 mg) every 8 h. After the recovery of organisms, the treatment was changed to intravenous penicillin G (200,000 U) every 6 h.

During the first 12 h after admission, the patient had several seizures which were controlled with phenobarbital and dilantin. The patient's febrile course returned to normal by day 4 of hospitalization.

Patient 2. A 2-week-old male infant was admitted to the emergency ward with a history of fever, constant crying, and an exaggerated startle reflex. There was no history of vomiting. His delivery and nursery stay were uncomplicated.

Physical examination revealed a crying infant with a rectal temperature of 37.2°C (101°F) and a very faint raised rash on the upper chest. The anterior fontanelle was soft. Cerebral spinal fluid examination revealed 6 cells per mm^3, 30% neutrophiles, and 70% lymphocytes; protein was 75 mg/dl, and glucose was 63 mg/dl. No organisms were seen on a Gram stain of the centrifuged specimen. The leukocyte count at admission was 24.0×10^3 cells per mm^3, hemoglobin was 11.9 g/dl, and the platelet count was 385×10^3 cells per mm^3, with 21% segmented neutrophiles, 43% bands, 22% lymphocytes, 7% monocytes, 2% eosinophiles, 1% basophiles, 1 atypical lymphocyte, and 3 metamyelocytes.

The patient was initially treated with intravenous ampicillin (200 mg per day) and intramuscular kanamycin (200 mg per day). Antibiotics were discontinued on day 7 of hospitalization, and the patient was discharged in good general condition. No medication was prescribed at the time of discharge.

Test procedure. The specimens collected for the blood cultures consisted of 6 ml of blood which was obtained by standard procedures utilizing strict aseptic techniques. 3 ml of the specimen was injected into aerobic (6B) and anaerobic (7C) BACTEC blood culture bottles (Johnston Laboratories, Inc., Cockeysville, Md.). These cultures were incubated at 35°C on an R2 New Brunswick Laboratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.) with constant agitation.

After 14 (patient 1) and 13 h (patient 2) of incubation, the cultures were Gram stained, confirming typical morphology and tinctoral characteristics of streptococci. Supernatant of the cultures were obtained by aspirating 1 ml. of the blood culture media and centrifuging at 2,500 rpm for 10 minutes. The supernatant was tested with the Phadebact Streptococcus Test kit groups A, B, C, and G (Pharmacia Diagnostics, Piscataway, N.J.) according to manufacturer's directions.

Trypticase soy blood agar base with 5% defibrinated sheep blood agar (BBL Microbiology Systems, Cockeysville, Md.) and chocolate agar in GC agar base with hemoglobin and IsoVitaleX (BBL Microbiology Systems) were inoculated with the blood cultures and incubated at 35°C in 7 to 10% CO_2 for 18 to 24 h. The resulting growth was Gram stained and tested with the Phadebact Streptococcus Test utilizing a 4-h incubation procedure (15). Confirming tests were also performed utilizing the hippurate hydrolysis test (11) and the CAMP test (5).

(i) Simulated blood cultures. Blood cultures were prepared from neonatal cord blood of groups A, B, AB, and O by injecting 3 ml of each blood group into separate aerobic (6B) BACTEC blood culture bottles. The blood culture bottles were then inoculated with *S. agalactiae*, *S. pyogenes*, beta-hemolytic streptococci Lancefield groups C and G, *S. faecalis*, and *S. pneumoniae*. These cultures were incubated at 37°C for 12 to 18 h and then tested by the procedures described below. Simulated blood cultures were also prepared with the above blood groups; however, inoculation with those organisms listed above was eliminated.

(ii) Treatment with dithiothreitol. The supernatant of the simulated blood cultures was heat inactivated at 56°C for 30 min and then treated with equal volumes of filter-sterilized dithiothreitol for 60 min. The dithiothreitol-treated supernatant was then tested with the Phadebact Streptococcus Test utilizing the supernatant test method. Dithiothreitol was prepared in glycine-buffered saline, pH 8.2, at a final concentration of 0.003 M (8, 17).

(iii) Treatment with soluble staphylococcal protein A. Soluble staphylococcal protein A was prepared by the method described by Forsgren and Sjöquist (6) with the omission of ethanol and trichloroacetic acid precipitation steps as described by Thirumoorthi and Dajani (16). The preparation contained 320 μg of protein A per ml. The supernatant of the simulated blood cultures was preincubated with the protein A solution by adding 1 drop of the protein A solution with 1 drop of the supernatant for 5 min before adding 2 drops of the streptococcal test reagent.

RESULTS

After 14 (patient 1) and 13 h (patient 2) of incubation, both patients' blood cultures demonstrated slight hemolysis. Gram stain results demonstrated typical morphology and tinctoral characteristics of streptococci. Testing of the supernatant fluid of the blood cultures with the Phadebact Streptococcus Test resulted in agglutination in the group B reagent. The agglutination was strong and easily read. No cross agglutination was observed.

After obtaining growth on blood agar, beta-hemolytic colonies were observed which were catalase negative and also exhibited the typical morphology and tinctoral characteristics of streptococci. Results of the 4-h serological grouping utilizing the Phadebact Streptococcus Test (15), the hippurate hydrolysis (11), and the CAMP test (5) confirmed those results obtained from the direct testing of the supernatant of the blood cultures.

Examination of the supernatant of the simulated blood cultures inoculated with *S. agalactiae* and streptococci of Lancefield groups A, C, and G revealed some weak nonspecific agglutination in all four blood groups when tested with the streptococcal test reagents. However, the agglutination observed with the supernatant of the simulated cultures was detectably stronger in the streptococcal test reagent corresponding to the organism used to inoculate that simulated blood culture. As indicated in the package insert, such reactions were considered positive for the group antigen which was the strongest.

Examination of the supernatant of the simulated blood cultures inoculated with *S. pneumoniae* and *S. faecalis* also revealed some weak nonspecific agglutination in all four blood groups. The simulated blood culture inoculated with *S. pneumoniae* produced detectably stronger agglutination in the group C streptococcal reagent, whereas the simulated blood culture inoculated with *S. faecalis* produced detectably stronger agglutination in the group A streptococcal reagent. Those simulated blood cultures of all four blood groups without the streptococcus inoculations also exhibited weak, nonspecific agglutination in groups A, B, C, and G streptococcal test reagents.

In an attempt to eliminate the nonspecific reactions, the supernatant of the simulated blood cultures was treated with dithiothreitol and soluble staphylococcal protein A. Treatment with dithiothreitol did not eliminate the weak, nonspecific agglutination observed. Treatment with soluble staphylococcal protein A did eliminate most of the nonspecific agglutination ob-

served in the simulated blood cultures inoculated with *S. agalactiae* and streptococci of Lancefield groups C and G. The nonspecific agglutination observed with *S. pneumoniae* and *S. faecalis* in the streptococcal test reagent groups C and A, respectively, could not be eliminated.

DISCUSSION

The coagglutination reagents now commercially available for grouping beta-hemolytic streptococci of groups A, B, C, and G provide the clinical laboratory with the capability of accurate and easy grouping of these streptococcal groups. The coagglutination method utilized as described here has many advantages over other methods used to identify group B streptococci. Lancefield grouping (12), hippurate hydrolysis (1), and the CAMP reaction (5) require 4 to 24 h of incubation after the suspected beta-hemolytic streptococci have been isolated on blood agar. Depending on the method used, this can involve a considerable amount of time, which may be critical when dealing with such a devastating infection in neonates.

By centrifuging blood cultures of neonates when streptococci are implicated by Gram stain and testing the cell-free supernatant, agglutination can easily be observed. The group-specific substance of the group B streptococci diffuses into the broth medium and is capable of being detected in the supernatant, according to Leland et al. (13), when 6×10^8 colony-forming units per ml are present.

Additional studies with simulated blood cultures revealed that nonspecific agglutination occurred when the blood cultures were inoculated with streptococci of groups A, B, C, or G. However, the agglutination was detectably stronger in the streptococcal test reagent corresponding to the organism used to inoculate the simulated blood culture. This nonspecific agglutination observed could not be eliminated with dithiothreitol, which is a sulfhydryl reducing agent. The rationale for this procedure is that dithiothreitol would reduce the sulfhydryl bonds present in immunoglobulin M (IgM) molecules that may be present, resulting in their inactivation.

When the supernatant was treated with soluble staphylococcal protein A, most of the nonspecific agglutination was eliminated yet had no effect on the specific agglutination reaction of the simulated blood cultures containing streptococcal group A, B, C, or G antigens. Protein A has been shown to bind to various subclasses of IgA, IgG, and IgM proteins (14), therefore, it is speculated that preincubation of the supernatant of the simulated blood cultures may have removed some of the IgG or IgA antibodies present without affecting the streptococcal antigens.

The agglutination observed in the streptococcal test reagents A and C when testing simulated blood cultures inoculated with *S. faecalis* and *S. pneumoniae*, respectively, may reflect expected false-positive reactions for groups A and C streptococci in clinical specimens. If the etiological agent is either *S. faecalis* or *S. pneumoniae*, misleading results may be obtained from utilizing this procedure. Although it is possible that these nonspecific reactions might occur in specimens from neonates with septicemia, the unusual occurrence of these and other streptococci (viridans group streptococci and nonenterococci) as the causative agents of neonatal septicemia implies that this type of reaction is unlikely to influence the usefulness of the procedure described for the detection of the group B streptococcal antigen.

Utilization of this technique when streptococcal infections are implicated in sepsis of neonates offers the clinical laboratory an easy to read, reliable, and rapid method for the direct serological grouping of group B streptococci from tbe supernatant of neonatal blood cultures.

ACKNOWLEDGMENTS

The assistance of the technical staff of the Microbiology Laboratory and the secretarial assistance of Debra Worrich are gratefully acknowledged.

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